NICEATM Draft Analysis:

Reduced Eye Hazard Labeling Resulting from Using Globally Harmonized System (GHS) Instead of Current U.S. Regulatory Classification Criteria

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Executive Summary

Recent analyses by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) reveal that up to 36% of substances currently classified and labeled as eye irritation hazards by U.S. hazard classification regulations would not be classified and labeled as eye hazards using United Nations Globally Harmonized System for the Classification and Labeling of Chemicals (GHS) eye irritation criteria (UN 2009[1]). Current U.S hazard classification regulations include the Federal Hazardous Substance Act (FHSA) regulations, used by the U.S. Consumer Product Safety Commission (CPSC) and the Occupational Safety and Health Administration (OSHA), and U.S. Environmental Protection Agency (EPA) hazard regulations. U.S. agencies are currently considering implementation of GHS criteria, and OSHA has recently proposed to adopt the GHS criteria to replace the current OSHA Hazard Communication Standard (HCS)(74 *FR* 50280[2]).

ICCVAM discovered the substantial differences in eye hazard labeling between the GHS and current U.S. classification systems while evaluating the validity of several *in vitro* methods proposed for regulatory ocular safety testing. NICEATM subsequently reviewed and analyzed two separate databases of *in vivo* eye irritation studies to assess the extent that using the GHS criteria would result in no hazard labeling for substances t currently labeled as eye hazards in the U.S.

The first ocular database evaluated for this analysis was constructed for chemicals used to prepare a 1999 OECD *Detailed Review Document* (DRD) *on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries* (**Appendix 1**[3]). This document proposed a potential harmonized classification scheme for eye hazards, and compared the impact on eye hazard labeling for existing national classification systems in Canada, EPA, EU, and FHSA. Careful review of the DRD reveals that using the GHS criteria resulted in no hazard labeling for up to 27% and 33% of substances labeled as eye hazards by current FHSA and EPA classification systems, respectively. This includes 76% of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS. Nonetheless, the scheme was subsequently adopted by GHS. The second database consisted of a public database of eye irritation studies for 149 chemicals, which revealed similar classification disparities. Using the GHS criteria resulted in no hazard labeling for up to 31% and 36% of substances currently labeled as eye hazards by FHSA and EPA classification systems, respectively.

[1]UN. 2009. Globally Harmonised System of Classification and Labelling of Chemicals (GHS). New York, Geneva: United Nations Publications.
[2]September 30, 2009 *Federal Register* (*FR*) notice (74 *FR* 50280): OSHA 29 CFR Parts 1910, 1915, and 1926 Hazard Communication: Proposed Rule.
[3]Available at http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294

NICEATM further characterized the nature, severity, and duration of eye injuries produced in these studies for the substances that will no longer be labeled as eye hazards using GHS criteria. Over 50% of these chemicals produced visible eye injuries expected to interfere with normal vision, including corneal opacity, corneal ulceration, and/or iritis (visible damage inside of the eye). Of these substances, 10% produced corneal opacity of a severity grade described as *easily defined translucent areas of the cornea that obscured the details of the iris* (i.e., corneal opacity score of 2/4). While all of the lesions were reversible, they persisted from 24 hours to seven days.

The high rate of reduced eye hazard labeling resulting from using the GHS criteria compared to U.S criteria is attributable to two important differences. First, the minimum number/proportion of animals with positive eye injury responses required for classifying a substance as an eye irritation hazard differs significantly. FHSA regulations classify substances as eye irritation hazards when as few as 22% (4/18) of animals produce positive eye injury responses, and EPA regulations classify substances as eye irritants when *any* single test animal exhibits a positive response, regardless of the number of animals tested. In contrast, GHS criteria require *at least* 67% (2/3) of animals tested to produce a positive response for classification as an eye irritant hazard. Secondly, there is a significant difference in the criteria that must be met for eye injuries to be considered a positive response. U.S. regulations (FHSA) consider it a positive response whenever the minimum severity is reached for any of the four types of ocular injuries at *any* of the three observation time points (24, 48, and 72 hours following test substance administration). In contrast, classification according to the GHS requires calculating the *average* severity across all three time points; this average score must meet or exceed the minimum severity level in order to be considered positive. Taken together, these two major differences account for the significant reduction in eye hazard labeling by GHS compared to current U.S. regulations.

The GHS incorporates the principle that *the level of protection offered to workers, consumers, the general public and the environment should not be reduced as a result of harmonizing the classification and labeling systems* (UN 2009). In order to adhere to the GHS principle of not reducing protection that could result from the significant reduction in labeling of eye hazards, GHS classification criteria are needed that can provide hazard labeling at least equivalent to that currently provided by current U.S. regulations. This paper summarizes the eye irritation hazard classification analyses and provides proposals for updating the GHS hazard criteria with an optional hazard category that could continue to provide the same level of hazard labeling and protection as current U.S. hazard regulations.

1.0 Background

Physical trauma or chemical burns due to contact with workplace or household products or chemicals result in about 125,000 household eye injuries each year and approximately 2,000 job-related eye injuries per day that require medical treatment[4,5]. In order to provide warnings to consumers and workers of the potential for chemicals and products to cause eye injuries, regulatory authorities require ocular safety testing to determine if substances may cause eye damage. Such testing characterizes the nature, duration, and severity of eye injuries in an animal model, and whether the injuries are reversible or permanent. Testing results are then used for hazard classification and labeling of eye injury potential according to relevant national and/or international classification systems. These classification systems are intended to warn users of the potential for substances to cause eye injuries, the precautions necessary to avoid injuries, and the immediate first-aid procedures that should be followed in the case of an accidental exposure.

Currently, OSHA's HCS uses the FHSA classification scheme (16 CFR 1500.42) to classify the ocular irritation hazard potential of regulated substances. The FHSA classification system is based on the proportion of animals that exhibit a minimum severity score for each of three areas of the eye (i.e., corneal ulceration and opacity, conjunctival redness and swelling, iritis) that occur during the first 72 hours following test substance administration, with observations recorded at 24, 48, and 72 hours (**Table 1-1**). **Appendix 2** provides the grading criteria for each of the types of ocular lesions. By comparison, classification according to the EPA scheme uses the same threshold for positive results in each tissue type, but has three severity categories, which are determined based on the maximum score for any of the three tissues in any one animal (**Table 1-2**).

Table 1-1 FHSA Classification System[1] (16 CFR 1500.42)

Positive Response for a Single Rabbit[1] (≥1 of the following at 24, 48, or 72 hr)	***In Vivo* Effect[2]**
• Corneal ulceration (other than a fine stippling) • Corneal opacity ≥ 1 • Iritis ≥ 1 • Conjunctival swelling and/or redness ≥ 2	First Test - If ≥4/6 animals are positive, the test is positive. If ≤1 animal is positive, the test is negative[3]. If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals. Second Test - If ≥3/6 animals are positive, the test is positive. If 0/6 are positive, the test is negative. If 1/6 or 2/6 animals are positive, the test is repeated using a different group of six animals. Third Test - Should a third test be needed, the test is positive if ≥1/6 animals are positive. If 0/6 are positive, the test is negative. **Note: Classification as an eye irritant hazard can result from as few as 22% of animals showing a positive response (e.g., 2/6+1/6+1/6=4/18).**

Abbreviations: CFR = U.S. Code of Federal Regulations; FHSA = Federal Hazardous Substances Act

[4] Available at: http://www.geteyesmart.org/eyesmart/injuries/home.cfm
[5] Available at: http://www.cdc.gov/niosh/topics/eye/

[1]The following scores are considered positive: CO or IR ≥ 1 or CC or CR ≥ 2. Therefore, CO or IR scores of 0 and CC or CR scores of ≤1 are considered cleared.
[2]In this evaluation, a test was also considered negative for 0/3, 0/4, or 0/5 positive animals in 3, 4, or 5-animal tests.

Table 1-2 EPA Classification System[1]

EPA Category	*In Vivo* Effect
I	Corrosive (irreversible) or corneal involvement or other eye irritation persisting for more than 21 days
II[2]	Corneal involvement or other eye irritation clearing[3] in 8 to 21 days
III[2]	Corneal involvement or other eye irritation clearing[3] in 7 days or less
IV	Minimal effects clearing[3] in less than 24 hours

Abbreviations: CC: conjunctival chemosis; CO: corneal opacity; CR: conjunctival redness; EPA = U.S. Environmental Protection Agency; IR: iritis
[1]At least 3 animals per test (one-animal screen for corrosive/severe irritants permitted).
Maximum score in any animal used for classification.
[2]The EPA currently bases classification decisions on the criteria presented in the EPA Label Review Manual (2003). However, these requirements differ from 40 CFR 156.62 (e.g., EPA Category III is based on no corneal involvement [EPA 2006]).
[3]The following scores are considered positive: CO or IR ≥ 1 or CC or CR ≥ 2. Therefore CO or IR scores of 0 and CC or CR scores of ≤1 are considered cleared.
Most severe response used for classification of substance.

In September 2009, OSHA proposed to modify the HCS to conform to the GHS system (74 *FR* 50280[6]). The GHS classification system is based primarily on the severity and timing of reversibility of effects using **mean** values for each endpoint (i.e., corneal opacity, conjunctival redness and swelling, iritis) based on observations assessed at 24, 48, and 72 hours following test substance administration (**Table 1-3**).

Table 1-3 GHS Classification System (UN 2009)

GHS Category	*In Vivo* Effect
I	≥ 1 animal with CO ≥ 4 at any time or ≥ 2 animals with **mean**[1] CO ≥ 3 or IR ≥ 1.5 or ≥1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which is not expected to reverse or does not fully reverse[2] within 21 days
2A	≥ 2 animals with **mean**[1] CO or IR ≥ 1 or CC or CR ≥ 2 which fully reverses[2] within 21 days
2B	≥ 2 animals with **mean**[1] CO or IR ≥ 1 or CC or CR ≥ 2 which fully reverses[2] within 7 days

Abbreviations: CC: conjunctival chemosis; CO: corneal opacity; CR: conjunctival redness; GHS = UN Globally Harmonized System; IR: iritis; UN = United Nations
[1]Mean value is calculated from grading at 24, 48, and 72 hours after instillation of the test material.
[2]Fully reversed requires a score = 0.

[6]September 30, 2009 Federal Register (FR) notice (74 FR 50280): OSHA 29 CFR Parts 1910, 1915, and 1926 Hazard Communication: Proposed Rule

To understand the potential impact of this change, NICEATM and ICCVAM evaluated 149 Draize rabbit eye tests in the ECETOC database (ECETOC 1998) for differences in classification of the test substances when comparing the GHS classification system to either the EPA classification system or the FHSA classification system.

NICEATM and ICCVAM also reviewed a 1999 OECD analysis of possible harmonized criteria for eye irritation and corrosion (which were ultimately adopted as the GHS criteria) that assessed the impact of the proposed criteria compared to current Canadian, EPA, EU, and FHSA labeling requirements based on 140 substances and 144 studies (4 repeat tests).

2.0 Overview of NICEATM and ICCVAM Analyses

To evaluate if and to what extent using the proposed HCS/GHS classification system might not identify substances as eye irritation hazards that would be classified as eye irritation hazards by FHSA and EPA criteria, NICEATM evaluated results from Draize rabbit eye test studies from two independent databases[7]: 1) 149 studies obtained from a publicly available database (ECETOC 1998[8]); and 2) 144 studies included in the *Detailed Review Document* (DRD) *on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries* (**Appendix 1**[9]).

All of the Draize eye test data used in these analyses are from studies that used no more than six animals. If the current FHSA criteria were applied to these studies, many substances could not be definitively classified for ocular hazard potential based on the results of the initial 6-animal test. To assign a definitive FHSA classification, these substances would require further testing in a second, and in some cases, a third 6-animal test. In order to establish a definitive FHSA classification for all substances, an analysis was first undertaken to determine the most appropriate minimum number of positive animals that could be used to assign an FHSA eye hazard label in such circumstances, and that would provide the same level of hazard labeling as current FHSA hazard classification regulations. This analysis (see **Appendix 3**) indicates that a minimum of one positive response out of three test animals would provide nearly equivalent labeling as current FHSA requirements. Based on this analysis, a threshold of ≥33% positive animals was used to assign a definitive classification for all substances included in the two databases.

3.0. Analysis of the ECETOC Eye Irritation Database

The ECETOC database was assessed to identify examples of substances classified based on Draize rabbit eye test results as GHS Not Classified, but FHSA Irritants or EPA Category I, II, or III irritants. Conversely, examples were also sought for substances classified as EPA Category IV or FHSA Not Labeled, but as GHS Category 1, 2A, or 2B.

[7]As noted in **Section 4.0**, the OECD database includes 24 substances that are also in the ECETOC database.
[8]ECETOC. 1998. Eye Irritation – Reference Chemicals Data Bank. Technical Report No. 48(2). European Centre for Ecotoxicology and Toxicology of Chemicals, Brussels.
[9]Available at http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294

3.1 Comparison of the FHSA and GHS Classification Systems

Where possible, NICEATM assigned FHSA and GHS hazard classifications for each substance in the ECETOC database[10]. Only substances that could be assigned a definitive FHSA and GHS classification were included, which yielded total of 122 or 134 substances included in the analysis, depending on whether the current FHSA criteria or a threshold of 33% positive animals, respectively was used. Among these substances, 69/122 (57%) and 81/134 (60%) were identified as ocular irritants by the FHSA using the current FHSA and 33% threshold criteria, respectively. NICEATM compared the FHSA ocular hazard classification of these substances with the classification that would be assigned by the GHS system. As indicated in **Table 3-1**, using the GHS criteria would result in no hazard labeling for up to 31% (25/81) of the ECETOC substances that are identified as ocular hazards by FHSA (see also **Appendix 4**). Conversely, there were no substances labeled as ocular hazards by the GHS that were not also labeled as hazards by the FHSA (**Table 3-1**).

Table 3-1 ECETOC Database: Substances Classified as Ocular Irritants Using FHSA Compared to Each GHS Ocular Hazard Category

FHSA Classification	No. ECETOC Substances Classified FHSA Irritants	GHS Classification (%)			
		1	2A	2B	NC
Irritant (33% threshold)	81	31/81 (38%)	18/81 (22%)	7/81 (9%)	25/81 (31%)
Irritant (16 CFR 1500.42)	69	31/69 (45%)	18/69 (26%)	7/69 (10%)	13/69 (19%)
Not Labeled (either criterion)	53	0/53 (0%)	0/53 (0%)	0/53 (0%)	53/53 (100%)

Abbreviations: CFR = U.S. Code of Federal Regulations; FHSA = U.S. Federal Hazardous Substances Act; GHS = UN Globally Harmonized System; NC = Not Classified

A closer look at the individual rabbit eye test data for the 25 FHSA eye irritants based on the 33% threshold that would not be labeled using GHS criteria reveals that 48% (12/25) of these substances produced corneal opacity and/or corneal ulceration, including seven that also produced iritis (visible evidence of tissue damage inside the eye, **Table 3-2**). Many of these substances (28% [7/25]) produced corneal opacity that extended beyond 48 hours after test substance administration (**Table 3-2**). **Table 3-2** also provides these data for the subset of 13 substances classified using the current FHSA criteria.

[10] The ECETOC database is comprised of 149 studies representing 145 substances. Three substances with duplicate studies and resulting in discordant hazard classifications among one or more of the hazard classification systems were excluded from these analyses (i.e., 1% benzalkonium chloride is GHS Category 1 or 2A; 5% triton X-100 is GHS Category 2A or 2B; xylene is EPA Category II or IV).

Table 3-2 ECETOC Database: Frequency, Type, and Severity of Ocular Lesions Among Substances Classified as FHSA Irritants, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

In Vivo Finding	No. of Substances (%)	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1] (%)
FHSA Classification Based on ≥33% Positive Animals		
Any CO Score ≥ 1	12/25 (48%)	10/12 (83%)
CO Score ≥ 1; Duration of 48 hr or more	7/25 (28%)	2/7 (29%)
CR or CC Score ≥ 2	22/25 (88%)	17/22 (68%)
CR or CC Score ≥ 2; Duration of 72 hr or more	5/25 (20%)	2/5 (40%)
Iritis; Visible inflammation inside the eye	7/25 (28%)	5/7 (71%)
Iritis; Duration of 48 hours or more	3/25 (12%)	-
FHSA Classification Based on 16 CFR 1500.42		
Any CO Score ≥ 1	10/13 (77%)	8/10 (80%)
CO Score ≥ 1; Duration of 48 hr or more	7/13 (54%)	2/7 (29%)
CR or CC Score ≥ 2	12/13 (92%)	12/12 (100%)
CR or CC Score ≥ 2; Duration of 72 hr or more	5/13 (38%)	2/5 (40%)
Iritis; Visible inflammation inside the eye	6/13 (46%)	5/6 (83%)
Iritis; Duration of 48 hours or more	3/13 (23%)	-

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; FHSA = U.S. Federal Hazardous Substances Act; HCS = OSHA Hazard Communication Standard; No. = number; OSHA = U.S. Occupational Safety and Health Administration
[1]The total number of animals in each test ranged from 3 to 6.

3.2 Comparison of the EPA and GHS Classification Systems

NICEATM also compared the ocular hazard classifications for the ECETOC substances based on EPA and GHS classification systems. Again, NICEATM attempted to assign EPA and GHS hazard classifications for each substance, and only substances that could be assigned a definitive EPA and GHS classification were included; a total of 134 substances were included in the analysis. Among these substances, 87/134 (65%) are identified as ocular irritants (i.e., EPA Category I, II, or III) by the EPA system. NICEATM compared the EPA ocular hazard classification of these substances with the classification that would be assigned by the GHS system. As indicated in **Table 3-3**, using the GHS criteria would result in no hazard labeling for 36% (31/87) of the ECETOC substances that are identified as ocular hazards by EPA (see also **Appendix 4**).

This includes 78% of currently labeled EPA Category III irritants (those causing eye injuries persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS (see **Table 3-4**). There were no substances labeled as ocular hazards by the GHS that were not also labeled as hazards by the EPA (**Table 3-4**).

Table 3-3 ECETOC Database: Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by GHS Classification Criteria

EPA Category I, II, or III	GHS Hazard Classification	No. of Substances (%)
87	1	36% (31/87)
	2A	21% (18/87)
	2B	8% (7/87)
	NC	36% (31/87)

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number

Table 3-4 ECETOC Database: Comparison of Substances Classified Using Each EPA and GHS Eye Hazard Category

EPA Classification	No. Substances	GHS Classification			
		1	2A	2B	NC
EPA I	28	27/27 (100%)	0/27 (0%)	0/27 (0%)	0/27 (0%)
EPA II	21	4/20 (20%)	14/20 (70%)	2/20 (10%)	0/20 (0%)
EPA III	42	0/40 (0%)	4/40 (10%)	5/40 (12%)	31/40 (78%)
EPA IV	47	0/47 (0%)	0/47 (0%)	0/47 (0%)	47/47 (100%)

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number

A closer look at the individual rabbit eye test data for the 31 EPA eye irritants that would not be labeled using GHS criteria reveals that 52% (16/31) of these substances produced corneal opacity and/or corneal ulceration, including eight (26% [8/31]) that extended beyond 48 hours after test substance administration (**Table 3-5**). A total of eight substances produced iritis (visible evidence of tissue damage inside the eye), seven of which also produced corneal opacity.

Table 3-5 ECETOC Database: Responses, Frequency, and Severity of Ocular Lesions Among 31 Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

In Vivo Finding	No. of Substances (%)	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1] (%)
Any CO Score ≥ 1	16/31 (52%)	10/16 (63%)
CO Score ≥ 1; Duration of 48 hours or more	8/31 (26%)	2/8 (25%)
CR or CC Score ≥ 2	25/31 (81%)	17/25 (68%)
CR or CC Score ≥ 2; Duration of 72 hr or more	5/31 (16%)	2/5 (40%)
Iritis; Visible inflammation inside the Eye	8/31 (26%)	5/8 (63%)
Iritis; Visible inflammation inside the Eye; Duration of 48 hours or more	3/31 (10%)	1/3 (33%)

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; HCS = OSHA Hazard Communication Standard; OSHA = U.S. Occupational Safety and Health Administration; No. = number
[1]The total number of animals in each test ranged from 3 to 6.

4.0 Analysis of the 1999 OECD Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries

During the development of possible harmonized criteria for eye irritation and corrosion hazard categories, the OECD coordinated preparation of a *Detailed Review Document* (DRD) *on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries* (**Appendix 1**[11]). This document provides a potential harmonized classification scheme along with a comparison to the impact on eye hazard labeling for several existing national classification systems (i.e., Canada, EPA, EU, and FHSA). The DRD provides clear and concise documentation of the extent that the potential harmonization scheme would significantly reduce the number of chemicals identified as eye irritation hazards compared to current U.S. (EPA and FSHA) requirements. The scheme proposed in the DRD was subsequently incorporated into the GHS (UN 2009). However, it should be noted that the DRD does not provide any conclusions or recommendations, but instead details comparisons of sensitivity offered by the existing classification systems and the proposed scheme. There is no discussion in the document as to why *not* labeling substances currently labeled as eye hazards by EPA and FHSA criteria could be construed as providing the same level of protection. Efforts to locate documentation of further consideration of the severe underlabeling of eye hazards and reduced protection that would result from using the GHS scheme compared to current U.S. requirements were unsuccessful.

The OECD DRD (hereafter, OECD database) includes Draize rabbit eye test data for 140 substances (144 studies - 4 repeat tests) that were obtained from five different sources: 1) ECETOC industrial chemicals (n=24); 2) EPA pesticide active ingredients (n=60); 3) EPA pesticide products (n=18); 4) EPA new industrial chemicals (n=27); and 5) German new industrial chemicals (n=11). NICEATM obtained the individual animal data from all 144 studies and assigned and EPA, FHSA, and GHS ocular hazard classification where possible. However, using the classification rules described in **Tables 1-1** to **1-3**, NICEATM was unable to assign a definitive classification (i.e., either irritant or not classified) for some of the substances (EPA, n=13; FHSA, n=14; GHS, n=19). Accordingly, there are some differences in the numbers of substances classified by NICEATM and those reported in the DRD (**see Appendix 5**). However, these differences did not result in substantive differences between NICEATM and the DRD database in the proportion of substances classified as irritants.

The OECD database was assessed to identify examples of substances classified based on Draize rabbit eye test results as GHS Not Classified, but FHSA Irritants or EPA Category I, II, or III irritants. Conversely, examples were also sought for substances classified as EPA Category IV or FHSA Not Labeled, but as GHS Category 1, 2A, or 2B.

4.1 Comparison of the FHSA and GHS Classification Systems

Where possible, NICEATM assigned FHSA and GHS hazard classifications for each substance in the OECD database. Only substances that could be assigned a definitive FHSA and GHS classification were included, which yielded total of 112 or 125 substances included in the analysis, depending on whether the current FHSA criteria or a

[11]Available at http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294

threshold of 33% positive animals, respectively was used. Among these substances, 85/112 (76%) and 95/125 (76%) were identified as ocular irritants by the FHSA using the current FHSA and 33% threshold criteria, respectively. NICEATM compared the FHSA ocular hazard classification of these substances with the classification that would be assigned by the GHS system. As indicated in **Table 4-1**, using the GHS criteria would result in no hazard labeling for up to 27% (26/95) of the OECD substances that are identified as ocular hazards by FHSA (see also **Appendix 6**). Conversely, there were no substances labeled as ocular hazards by the GHS that were not also labeled as hazards by the FHSA (**Table 4-1**).

Table 4-1 OECD Database: Substances Classified as Ocular Irritants Using FHSA Compared to Each GHS Ocular Hazard Category

FHSA Classification	No. OECD Substances Classified FHSA Irritants	GHS Classification (%)			
		1	2A	2B	NC
Irritant (33% threshold)	95	38/95 (40%)	22/95 (23%)	9/95 (9%)	26/95 (27%)
Not Labeled (33% threshold)	30	0/30 (0%)	0/30 (0%)	0/30 (0%)	30/30 (100%)
Irritant (16 CFR 1500.42)	85	38/85 (45%)	22/85 (26%)	9/85 (10%)	16/85 (19%)
Not Labeled (16 CFR 1500.42)	29	0/29 (0%)	0/29 (0%)	0/29 (0%)	29/29 (100%)

Abbreviations: CFR = U.S. Code of Federal Regulations; FHSA = U.S. Federal Hazardous Substances Act; GHS = UN Globally Harmonized System; OECD = Organisation for Economic Co-operation and Development; NC = Not Classified; No. = number

A closer look at the individual rabbit eye test data for the 26 FHSA eye irritants based on the 33% threshold that would not be labeled using GHS criteria reveals that 46% (12/26) of these substances produced corneal opacity and/or corneal ulceration, including twelve that also produced iritis (visible evidence of tissue damage inside the eye, **Table 4-2**). Many of these substances (27% [7/26]) produced corneal opacity that extended beyond 48 hours after test substance administration (**Table 4-2**). **Table 4-2** also provides these data for the subset of 16 substances classified using the current FHSA criteria.

Table 4-2 OECD Database: Frequency, Type, and Severity of Ocular Lesions Among Substances Classified as FHSA Irritants, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

In Vivo Finding	No. of Substances (%)	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1] (%)
FHSA Classification Based on ≥33% Positive Animals		
Any CO Score ≥ 1	12/26 (46%)	8/12 (67%)
CO Score ≥ 1; Duration of 48 hr or more	7/26 (27%)	6/7 (86%)
CR or CC Score ≥ 2	22/26 (85%)	20/22 (91%)
CR or CC Score ≥ 2; Duration of 72 hr or more	4/26 (15%)	4/4 (100%)
Iritis; Visible inflammation inside the eye	12/26 (46%)	6/12 (50%)
Iritis; Duration of 48 hours or more	2/26 (8%)	1/2 (50%)
FHSA Classification Based on 16 CFR 1500.42		
Any CO Score ≥ 1	8/16 (50%)	5/8 (62%)
CO Score ≥ 1; Duration of 48 hr or more	5/16 (31%)	4/5 (80%)
CR or CC Score ≥ 2	16/16 (100%)	15/16 (94%)
CR or CC Score ≥ 2; Duration of 72 hr or more	3/16 (19%)	2/3 (67%)
Iritis; Visible inflammation inside the eye	8/16 (50%)	5/8 (62%)
Iritis; Duration of 48 hours or more	2/16 (12%)	2/2 (100%)

Abbreviations; CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; FHSA = U.S. Federal Hazardous Substances Act
[1]The total number of animals in each test ranged from 3 to 6.

4.2 Comparison of the EPA and GHS Classification Systems

NICEATM also compared the ocular hazard classifications for the ECETOC substances based on EPA and GHS classification systems. Again, NICEATM attempted to assign EPA and GHS hazard classifications for each substance, and only substances that could be assigned a definitive EPA and GHS classification were included; a total of 122 substances were included in the analysis. Among these substances, 99/122 (81%) are identified as ocular irritants (i.e., EPA Category I, II, or III) by the EPA system. NICEATM compared the EPA ocular hazard classification of these substances with the classification that would be assigned by the GHS system. As indicated in **Table 4-3**, using the GHS criteria would result in no hazard labeling for 33% (33/99) of the ECETOC substances that are identified as ocular hazards by EPA. This includes 76% (31/41) of currently labeled EPA Category III irritants (those causing eye injuries

persisting for 24 hours to 7 days) that would not require hazard labeling using the GHS (see **Table 4-4** and **Appendix 6**). There were no substances labeled as ocular hazards by the GHS that were not also labeled as hazards by the EPA (**Table 4-4**).

Table 4-3 OECD Database: Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by GHS Classification Criteria

EPA Category I, II, or III	GHS Hazard Classification	No. of Substances (%)
99	1	36% (36/99)
	2A	22% (22/99)
	2B	8% (8/99)
	NC	33% (33/99)

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number

Table 4-4 OECD Database: Comparison of Substances Classified Using Each EPA and GHS Eye Hazard Category

EPA Classification	No. Substances	GHS Classification			
		1	2A	2B	NC
EPA I	36	35/36 (97%)	1/36 (3%)	0/36 (0%)	0/36 (0%)
EPA II	22	1/22 (4%)	18/22 (82%)	1/22 (4%)	2/22 (9%)
EPA III	41	0/41 (0%)	3/41 (7%)	7/41 (17%)	31/41 (76%)
EPA IV	23	0/23 (0%)	0/23 (0%)	0/23 (0%)	23/23 (100%)

Abbreviations: EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; NC = Not Classified; No. = number

A closer look at the individual rabbit eye test data for the 33 EPA eye irritants that would not be labeled using GHS criteria reveals that 39% (13/33) of these substances produced corneal opacity and/or corneal ulceration, including seven (21% [7/33]) that extended beyond 48 hours after test substance administration (**Table 4-5**). A total of twelve substances produced iritis (visible evidence of tissue damage inside the eye), six of which also produced corneal opacity.

Table 4-5 ECETOC Database: Responses, Frequency, and Severity of Ocular Lesions Among 33 Substances Classified in the U.S. as Ocular Hazards Using the EPA Hazard Category Criteria, but Not Classified as Ocular Hazards by the Proposed HCS and Current GHS Classification Criteria

In Vivo Finding	No. of Substances (%)	No. of Substances Where More than One Animal Exhibited the *In Vivo* Finding[1] (%)
Corneal Opacity/Ulceration Score ≥ 1	13/33 (39%)	8/13 (62%)
CO Score ≥ 1; Duration of 48 hours or more	7/33 (21%)	6/7 (86%)
CR or CC Score ≥ 2	28/33 (85%)	20/28 (71%)
CR or CC Score ≥ 2; Duration of 72 hr or more	6/33 (18%)	2/6 (33%)
Iritis; Visible inflammation inside the Eye	12/33 (36%)	6/12 (50%)
Iritis; Visible inflammation inside the Eye; Duration of 48 hours or more	2/33 (6%)	1/2 (50%)

Abbreviations: CC = conjunctival chemosis; CO = corneal opacity; CR = conjunctival redness; EPA = U.S. Environmental Protection Agency; GHS = UN Globally Harmonized System; HCS = OSHA Hazard Communication Standard; OSHA = U.S. Occupational Safety and Health Administration; No. = number
[1]The total number of animals in each test ranged from 3 to 6.

5.0 Summary of Analyses

These results from two independent databases of Draize rabbit eye test results are consistent and indicate that a significantly greater proportion of substances causing eye irritation, including some substances producing eye injuries lasting more than seven days (EPA Category II), will not be labeled using the GHS criteria. Taken together, these data indicate that the GHS hazard classification criteria will significantly reduce eye hazard labeling compared to that provided by current HCS/FHSA regulations. *Of greatest concern is that the proposed HCS and current GHS classification criteria will not identify many substances as eye irritants that produce significant ocular damage, including extended corneal opacity which can result in visual impairment and internal ocular inflammation.*

6.0 Possible Options for GHS Hazard Categories for Classification and Labeling of Reversible Eye Irritation

Paragraph 1.1.1.6 of the GHS states that during the development of the GHS, *The requirements of [the U.S., Canada, EU, and] other countries were also examined as the work developed, but the primary task was to find ways to adopt the best aspects of these existing systems and develop a harmonized approach. This work was done based on agreed principles of harmonization that were adopted early in the process: (a) the level of protection offered to workers, consumers, the general public and the environment should not be reduced as a result of harmonizing the classification and labeling systems...*" (UN 2009[12]).

The current GHS criteria for classification of reversible ocular irritants (Category 2) involve scoring three-animal tests for eye lesions (corneal opacity, iritis, conjunctival redness and chemosis) on days 1, 2, and 3 (see also **Table 6-1**). A mean score is calculated for each animal using the three daily observation scores and a Category 2 is assigned for those substances that induce any of the following **mean** animal scores in at least **two** animals: corneal opacity or iritis ≥1 or conjunctival redness or chemosis ≥ 2 that persists beyond seven days, but reverses within 21 days. Any substances not meeting this requirement would not be labeled as an ocular hazard. An optional Category (2B) is also provided for regulatory authorities to subcategorize Category 2 eye irritants as mild irritants if positive responses reverse by day 7.

Given the large number of substances that are labeled as eye hazards by current U.S. regulatory classification systems (FHSA and EPA), but that are Not Labeled as eye hazards by the current GHS classification system, NICEATM and ICCVAM performed technical analyses to support three optional GHS hazard categories that would achieve the GHS principle stated above. Countries and regulatory authorities could then choose to adopt the optional categories as necessary in order not to reduce the protection compared to the current level of protection afforded by the respective national or agency classification regulations. Each of the three proposals below provide classification criteria for a three-animal test that will provide the same level of hazard labeling as current FHSA, HCS, and EPA hazard classification regulations. The proposals are as follows:

- **Proposal #1** (**Table 6-1**): Current GHS Category 2 is unchanged; an optional Category 3 is included for those countries that need such a category to maintain the current level of hazard labeling.
    - Assign Category 3 based on positive ocular lesions obtained in any animal at any time point.

      Category 3A: Any lesions that reverse within 21 days.

      Category 3B: Any lesions that reverse within 7 days.

[12]UN. 2009. Globally Harmonised System of Classification and Labelling of Chemicals (GHS). New York, Geneva: United Nations Publications.

- **Proposal #2** (**Table 6-2**): Current GHS Category 2A is unchanged; Current GHS Category 2B criteria changed based on ocular lesions in at least one animal at any time point, and that reverses within 21 days.
  - An optional Category 2C would be used when ocular lesions in Category 2B reverse within 7 days.
- **Proposal #3** (see **Table 6-3**): Modify the current GHS Category 2A and 2B.
  - Assign category based on ocular lesions obtained in at least one animal at any of the three time points.

Table 6-4 provides a comparison of these three proposals to the current GHS hazard categories. In conclusions, each of these three proposals will provide GHS classification criteria that can be used to maintain the same level of labeling and protection afforded by current EPA and FHSA hazard criteria regulations.

Table 6-1: Proposal #1 – Addition of an Optional Category 3

Category	Current GHS	Proposal #1
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 21 days	Same as current GHS
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days	Same as current GHS
3A (optional)		≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which fully reverses within 21 days
3B (optional)		≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days

CC: conjunctival chemosis; CO: corneal opacity; CR: conjunctival redness; IR: iritis
[1]Mean values are calculated over 24 to 72 hours.

Table 6-2: Proposal #2 – Modify the Optional Category 2B and Add Another Optional Category

Category	Current GHS	Proposal #2
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 21 days	Same as current GHS
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days
2C (optional)		>1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days

CC: conjunctival chemosis; CO: corneal opacity; CR: conjunctival redness; IR: iritis
[1]Mean values are calculated over 24 to 72 hours.

Table 6-3: Proposal #3 – Categories Based on Individual Animal Scores Instead of Mean Animal Scores

Category	Current GHS	Proposal #3
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 21 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 7 days

CC: conjunctival chemosis; CO: corneal opacity; CR: conjunctival redness; IR: iritis
[1]Mean values are calculated over 24 to 72 hours.

Table 6-4: Comparison of Current GHS Categories to Possible Optional Categories

Category	Current GHS	Proposal #1	Proposal #2	Proposal #3
2A	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 21 days	Same as current GHS	Same as current GHS	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days
2B (optional)	≥ 2 animals with mean[1] CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days	Same as current GHS	≥ 1 animals with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 21 days	≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 at any time which reverses within 7 days
2C (optional)			>1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days	
3A (optional)		≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which fully reverses within 21 days		
3B (optional)		≥ 1 animal with CO or IR ≥ 1 or CC or CR ≥ 2 which reverses within 7 days		

CC: conjunctival chemosis; CO: corneal opacity; CR: conjunctival redness; IR: iritis
[1]Mean values are calculated over 24 to 72 hours.

Appendix 1

OECD SERIES ON TESTING AND ASSESSMENT – NUMBER 14: Detailed Review Document on Classification Systems for Eye Irritation/Corrosion in OECD Member Countries. ENV/JM/MONO(99)4. Available at http://www.olis.oecd.org/olis/1999doc.nsf/LinkTo/env-jm-mono%2899%294

Appendix 2

Grades for Ocular Lesions[13]

Cornea	**Score**
Opacity: Degree of density (area most dense taken for reading). No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	*1
Easily discernible translucent area, details of iris slightly obscured	*2
Nacrous area, no details or iris visible, size of pupil barely discernible	*3
Opaque cornea, iris not discernible through the opacity	*4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	*1
No reaction to light, hemorrhage, gross destruction (any or all of these)	*2
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae, excluding cornea and iris).	
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	*2
Diffuse beefy red	*3
Chemosis (refers to lids and/or nictitating membranes)	
No swelling	0
Any swelling above normal (includes nictitating membranes)	1
Obvious swelling with partial eversion of lids	*2
Swelling with lids about half closed	*3
Swelling with lids more than half-closed	*4

*Starred figures indicate positive grades.

[13] Reproduced from EPA. 1998. Health Effects Test Guideline, OPPTS 870.2400 Acute Eye Irritation. EPA 712-C-98-195. U.S. Environmental Protection Agency: Washington, DC.

Appendix 3

Consideration of the Minimum Number of Animals with Positive Eye Injury Responses Required for Classification of a Chemical as an Eye Irritation Hazard

Abstract

Current regulations under the U.S. Federal Hazardous Substances Act, specify a classification system based on using up to three sequential tests for each substance, with six-animals used per test and decisions on further sequential testing based on the number of positive responses observed in each test. However, current best practices for eye irritation/corrosion testing involve sequential testing of up to a total of three animals. Therefore, an analysis was undertaken to determine the most appropriate minimum number of positive animals that should be required for FHSA eye hazard labeling based on results from a three-animal test. Three different classification strategies were compared and the frequency at which each would identify substances as ocular irritants. A number of different response rates and the resulting classification that would be assigned by each strategy were also compared. These analyses indicate that using a criterion of at least one positive animal in a three-animal test as the basis for classification as an eye irritation hazard would be considered at least as protective as the current FHSA testing requirements and criteria that use 6-18 animals. Accordingly, a proposal is presented that includes classification criteria for a three-animal test that will provide the same or more protective level of hazard labeling as current FHSA requirements, while using up to 83% fewer animals.

Introduction

Physical trauma or chemical burns due to contact with workplace or household products or chemicals result in about 125,000 household eye injuries each year and approximately 2,000 job-related eye injuries per day that require medical treatment. In order to provide warnings to consumers and workers of the potential for chemicals and products to cause eye injuries, regulatory authorities require ocular safety testing to determine if substances may cause eye damage. Testing results are then used for hazard classification and labeling of eye injury potential according to relevant national and/or international classification systems. These classification systems are intended to warn users of the potential for substances to cause eye injuries, the precautions necessary to avoid injuries, and the immediate first-aid procedures that should be followed in the case of an accidental exposure.

The guidelines for classification of ocular irritation hazard potential for substances regulated under the Federal Hazardous Substances Act (FHSA, FHSA 2005) are described in 16 CFR 1500.42 (CPSC 2003). The FHSA system is based on the severity of effects for each endpoint (i.e., corneal ulceration and opacity, conjunctival redness and swelling, iritis) that occur during the first 72 hours following test substance administration with observations recorded at 24, 48, and 72 hours (**Table 1**).

Table 1: FHSA Classification System[1] (16 CFR 1500.42) [1,2]

Positive Response for a Single Rabbit[3] (≥1 of the following at 24, 48, and/or 72 hr)	*In Vivo* Effect
• Corneal ulceration (other than a fine stippling) • Corneal opacity ≥ 1 • Iritis ≥ 1 • Conjunctival swelling and/or redness ≥ 2	First Test - If ≥4/6 animals are positive, the test is positive. If ≤1 animal is positive, the test is negative. If 2/6 or 3/6 animals are positive, the test is repeated using a different group of six animals. Second Test - If ≥3/6 animals are positive, the test is positive. If 0/6 are positive, the test is negative. If 1/6 or 2/6 are positive, the test is repeated using a different group of six animals. Third Test - Should a third test be needed, the test is positive if ≥1/6 animals are positive. If 0/6 are positive, the test is negative.

Abbreviations: CC = conjunctival chemosis; CFR = Code of Federal Regulations; CO = corneal opacity; CR = conjunctival redness; FHSA = Federal Hazardous Substances Act; IR = iritis
[1]For the FHSA Classification System (2005), the testing guidelines and associated regulations are included in 16 CFR 1500.42 (CPSC 2003).
[2]At least three animals per test (one animal screen for corrosive/severe irritants permitted). Maximum score in any animal used for classification.
[3]The following scores are considered positive: CO or IR ≥1 or CR or CC ≥2. Therefore, CO and IR scores of 0 or CR and CC scores ≤1 are considered negative.

Current best practices for eye irritation/corrosion testing involve sequential testing of up to a total of three animals (e.g., OECD TG 405, OECD 2002), given that statistical analyses demonstrated that results from rabbit eye tests using only 3 animals consistently agreed with the outcome of a 6-animal test (DeSousa et al. 1984; Talsma et al. 1988; Springer et al. 1993). However, as indicated in **Table 1**, the current FHSA regulations for ocular hazard classification and labeling are based on using up to three sequential tests for each substance, with six animals used per test and decisions on further sequential testing based on the number of positive responses in each test. Therefore, there is a need to develop criteria for hazard classification and labeling under the FHSA that could be applied to results from a 3-animal test, while providing the same level of protection achieved by the more extensive testing strategy. Accordingly, an analysis was undertaken to determine the most appropriate minimum number of positive animals that should be required for FHSA eye hazard labeling if only a three animal test is used.

Methods

In order to determine the optimal number of positive animals that would require FHSA hazard classification and labeling, the current FHSA requirements were evaluated to determine the minimum number of animals that would be required under the sequential testing strategy to assign a definitive classification (**Table 2**). The weakest possible response that is considered positive by the FHSA classification system is 22%

(2/6+1/6+1/6 or 4/18 or 22%). However, it is possible for an even higher positive response rate (3/6+2/6+0/6 or 5/18 or 28%) to be considered negative according to the FHSA system (see **Table 2**). Ideally, a classification system should not produce such internal inconsistencies. For this evaluation, the current sequential testing strategy used to assign an FHSA classification, which could use up to 18 animals, is designated as Strategy 1.

Because all of the Draize eye test data used in the NICEATM analyses are from studies that used no more than six animals, NICEATM also evaluated a potential criterion where a minimum of one or more positives out of three animals (i.e., ≥33% positive animals) would be required to assign an irritant classification. For this evaluation, the ≥1/3 threshold is designated as Strategy 2.

Table 2 Number of Animals Required to Assign an Irritant Classification According to the Current FHSA Requirements[1]

Positive Test Criteria for "Irritant" Classification	Positive Animals	Positive Animals	Positive Animals	Positive Animals	Positive Animals	Positive Animals
First Test	≥4/6	2/6 or 3/6	3/6	3/6	2/6	2/6
Second Test	-	≥3/6	2/6	1/6	2/6	1/6
Third Test	-	-	≥1/6	≥1/6	≥1/6	≥1/6
Minimum Number of Positive Animals for Irritant	**4/6 (67%)**	**5/12 (42%)**	**6/18 (33%)**	**5/18 (28%)**	**5/18 (28%)**	**4/18 (22%)**
Maximum Number of Positives for Not Labeled	**1/6 (17%)**	**2/12 (17%)**	**5/18 (28%)**	**4/18 (22%)**	**4/18 (22%)**	**3/18 (17%)**

[1]For the FHSA Classification System (2005), the testing guidelines and associated regulations are included in 16 CFR 1500.42 (CPSC 2003).

By comparison, the United Nations Globally Harmonized System of Classification and Labeling of Chemicals (GHS, UN 2007) is based on a three-animal test where at least 67% (2/3) of animals tested must produce a positive response[14] in order to assign an irritant (i.e., GHS Category 2). Therefore, a threshold of 2/3 (67%) is designated as Strategy 3, but is based on the same criterion as Strategies 1 and 2, that a positive is based on a positive response at any of the three observation points, rather than the mean of the response over all three timepoints as currently required by GHS classification system.

Results

In order to compare the three strategies with regard to the frequency at which each strategy would identify substances as ocular irritants, a number of different response rates

[14] Based on mean values for each test animal calculated from grading at 24, 48, and 72 hours following test substance administration.

and the resulting classification that would be assigned by each strategy were compared. As indicated in **Table 3**, Strategy 3 will identify far less irritants than either Strategy 1 (current FHSA requirements) or Strategy 2. For example, if for a given substance half of all animals tested on average produce a positive response, then Strategy 3 only has a 50% chance of calling that substance an eye irritant, compared with 88% for Strategies 1 or 2.

Table 3: Percentage of Substances That Would Be Labeled as Ocular Irritants Based on Three Different Evaluation Strategies

Underlying Response Rate	Percentage of Substances That Would Be Labeled as Ocular Irritants		
	Strategy 1 Current FHSA[1]	Strategy 2 ≥1/3 positive animals	Strategy 3 ≥2/3 positive animals
20%	20.4%	48.8%	10.4%
40%	72.6%	78.4%	35.2%
50%	87.9%	87.5%	50.0%
75%	>99%	98.4%	84.3%

[1]For the FHSA Classification System (2005), the testing guidelines and associated regulations are included in 16 CFR 1500.42 (CPSC 2003).

To illustrate the calculations summarized in **Table 3**, suppose that on average 20% of all animals tested will produce a positive response. Using the current FHSA requirements, a negative classification could result in either the first, second, or third tests. Based on the binomial distribution, the likelihood of observing 0/6, 1/6, 2/6, 3/6, or >3/6 positives is 0.262, 0.393, 0.246, 0.082, and 0.017 respectively. The probability that the first test will produce a negative classification is simply the sum of the likelihood of observing 0/6 and 1/6 positive responses or 0.655. Thus, 65.5% of the time, no further testing would be necessary, and the substance would not be labeled.

A second test would be needed if the first test positive outcome rate was either 2/6 or 3/6 (likelihood=0.328). Then the second test would result in a negative classification if 0/6 positive responses were observed, making the likelihood of a negative classification by the second test (0.328)(0.262) or .086 (8.6%).

The third test would be needed if the second test showed 1/6 or 2/6 positives responses, which would occur with a likelihood of 0.639. Then the third test would produce a negative classification if 0/6 positive responses were observed. Thus, the likelihood that a negative classification will result from the third test is simply (0.328)(0.639)(0.262) or 0.055 (5.5%). Adding these three probabilities results in the overall likelihood of a negative classification of 0.655+0.086+0.055 or 0.796 (79.6%), and thus the likelihood of a positive classification by subtraction is 1-0.796 or 0.204 (20.4%; see Table 3).

These calculations are much simpler for Strategies 2 and 3. The likelihood of a positive classification using Strategy 2 is just 1 minus the likelihood of observing 0/3 positives or 1-(0.8)(0.8)(0.8) or 0.488 (48.8%). For Strategy 3, a positive response rate of 1/3

(likelihood =0.384) would also lead to a negative classification, making the overall likelihood of a positive classification for Strategy 3: 0.488-0.384 or 0.104 (10.4%).

Three important results are evident from **Table 3**: (i) Even though it uses fewer animals, Strategy 2 is more powerful than the current FHSA requirements for detecting positive response rates of 20-40%; (ii) Strategy 3 has low power in all cases considered; and (iii) Strategy 2 is the only strategy that always regards a single positive outcome as indicating an irritant response. For example, the current FHSA requirements may have as many as five animals showing a positive response, and yet the substance is still not considered an irritant (**Table 2**), while Strategy 3 considers a positive response rate of 33% (1/3) to not be indicative of an irritant response.

Discussion

Given that many national and international ocular safety testing guidelines now require only three animals, it is unlikely that users are conducting ocular safety tests as described in the current FHSA requirements and thus an update to these hazard classification guidelines appears in order. These analyses can be used to establish criteria that are needed to maintain the same level of eye hazard labeling as the current FHSA and using a three-animal test. The results detailed herein indicate that the minimum number of animals with a positive response in a three-animal test required for classification as an eye hazard that would be considered at least equivalent to the current FHSA requirements is one of three positives (Strategy 2) rather than two out of three positives (Strategy 3).

It should also be emphasized that Strategy 3 approximates the GHS classification system with one important exception: it assumes that any positive response at any time point is used for a positive animal. In contrast, the GHS classification system uses mean values for each test animal calculated from grading at 24, 48, and 72 hours following test substance administration. Therefore, the criteria for a positive response under the proposed GHS system requires an even higher threshold for identifying an irritant than does Strategy 3, and one can assume that the actual differences between Strategy 1 or 2 and Strategy 3 developed based on mean calculations are even greater than presented in Table 3. For this reason, the criteria for a positive animal response provided in the current FHSA eye hazard regulations (i.e., a positive score at any time point during the observation period) are preferred for any revised classification system, rather than a mean value calculated from three time points (as in the GHS system).

Applying these rules to revised FHSA requirements will substantially reduce the number of animals required to assign a definitive classification for ocular hazard potential of substances and materials that are regulated under the FHSA classification system. Creating hazard classification criteria that are based on a three-animal test, rather than the currently required sequential six-animal test that could require up to 18 animals, would have an immediate impact on reducing the number of animals required for ocular safety testing by up to six-fold.

References

CPSC. 2003. Test for Eye Irritation. 16 CFR 1500.42. Office of the Federal Register: Washington, DC:U.S. Government Printing Office.

DeSousa et al. 1984. Statistical consequences of reducing the number of rabbits utilized in eye irritation testing: data on 67 petrochemicals. Toxicol App Phamacol 76:234-242.

FHSA. 2005. Federal Hazardous Substances Act (FHSA). Public Law 86-613. [74 Stat.372].

OECD. 2002. Test Guideline 405, Acute Eye Irritation/corrosion, adopted April 24, 2002. In OECD Guidelines for Testing of Chemicals. Paris:OECD.

Springer et al. 1993. Number of animals for sequential testing. Food Chem Toxicol. 31:105-109.

Talsma et al., 1988. Reducing the number of rabbits in the Draize eye irritancy test: a statistical analysis of 155 studies conducted over 6 years. Fund App Toxicol. 10:146-153.

United Nations. 2009. Globally Harmonized System of Classification and Labelling of Chemicals (GHS): Third Revised Edition. New York:United Nations, Economic Commission for Europe.